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Association of Endotoxin and Allergens with Respiratory and Skin Symptoms: A Descriptive Study in Laboratory Animal Workers

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Abstract

Background: In laboratory animal work, allergens are classically considered to play a prominent role in generation of respiratory and skin symptoms. However, recent development may have changed working conditions and require an updating of preventive measures.

Objective: In workers exposed to a range of animals besides laboratory mice and rats the relative role of endotoxin, irritants, and allergens in symptom generation was assessed for updating preventative measures and health surveillance.

Methods: Eligible workers were recruited from university units in which exposure to rats and/or mice, occurrence of respiratory and/or skin symptoms, and/or a history of animal bites had been reported. Exposure to endotoxin and rat and mouse allergen was assessed (71 half-day personal samples). 'Symptomatic' was defined by work-related ocular, nasal, respiratory, or skin symptoms. A concentration of specific IgE against rat or mouse (e87 and e88) ≥0.35 kU/l defined sensitization. Sensitivity analyses examined the effect of alternative exposure indicators and definitions of 'sensitized' and 'symptomatic'.

Results: From 302 eligible workers, 177 participated. There were 121 and 41 workers in the asymptomatic and non-sensitized and symptomatic but non-sensitized group, respectively. Eight subjects were symptomatic and sensitized. Six sensitized subjects were asymptomatic. One participant could not be assigned to a subgroup. Airborne endotoxin and allergen concentrations were mostly below 20 EU m^{-3} or the detection limit, respectively. Clinical history showed that irritants and sensitizers

other than mouse/rat allergen or endotoxin were a major cause of symptoms. Results were sensitive to the selected exposure indicator and the definition of 'symptomatic'.
Conclusions: Health surveillance programs need to be adapted to include a larger range of allergens and pay more attention to irritants.

Keywords: asthma; irritants; laboratory animal; rhinitis; sensitizers

Introduction

Laboratory animal (LA) allergy is a well-recognized occupational disease. Classically, the disease develops within 1–4 years after starting work and is more likely with high exposure to animal allergens, atopy, and sensitization or allergy to pet cats or dogs. It has also been suggested that variability of exposure levels, IgG or IgG4 concentrations, and genetic variants may be risk factors (Pacheco *et al.*, 2008; Nicholson *et al.*, 2010; Pacheco *et al.*, 2010; Peng *et al.*, 2011; Phipatanakul *et al.*, 2012; Jones *et al.*, 2014; Jones, 2015; Feary and Cullinan, 2016). Risk assessment is currently difficult because of uncertainties surrounding the cause of disease and the level of exposure.

With respect to cause of disease, a large proportion of symptomatic workers are not sensitized (Lieutier-Colas *et al.*, 2002; Pacheco *et al.*, 2003; Schmid *et al.*, 2009; Samadi *et al.*, 2012). Indeed, the incidence of occupational asthma defined by work-related chest symptoms was about 2–4 cases × 100 person-years, whereas the incidence of allergic asthma defined by symptoms and positive prick tests was much lower (0.4–1.6 cases × 100 person-years) (Folletti *et al.*, 2008). Similar findings were reported for occupational rhinitis (7–11 versus 2–5 × 100 person-years) (Folletti *et al.*, 2008). The large difference between the prevalence of symptomatic workers and of symptomatic workers with specific sensitization to rat or mouse suggests a role for irritants (Kacergis *et al.*, 1996; Kogevinas *et al.*, 2007) or allergens not looked for in the study, e.g. sensitization to storage or house dust mites (Hollander *et al.*, 1996; Ruoppi *et al.*, 2005), other animal allergens (Botham *et al.*, 1987), powdered latex gloves, drugs, or anesthetics.

Besides the aforementioned agents, the role of pathogen-associated molecular patterns (PAMPs) has been suggested. Although endotoxin remains an important agent assumed to cause activation of the innate immune system and neutrophilic asthma (Haldar and Pavord, 2007; Doreswamy and Peden, 2011; Poole and Romberger, 2012) its clinical relevance in LA workers is currently conflicting (Lieutier-Colas *et al.*, 2002; Pacheco *et al.*, 2003; Freitas *et al.*, 2016). The role of peptidoglycans, gram-positive bacteria cell wall components, (1→3)-β-D-glucans, and fungi have hardly been studied in LA workers.

With respect to exposure levels of LA workers to allergens and PAMPs, the situation may be rapidly changing because genetically modified mice have largely supplanted rats, individually ventilated cages (IVC) have been introduced in place of traditional open cages, and more attention is paid to personal protective equipment (Jones, 2015; Feary and Cullinan, 2016). However, some tasks still cause high exposures (e.g. animal handling or cage dumping) (Curtin-Brosnan *et al.*, 2010; Glueck *et al.*, 2012). Hence, it is uncertain whether an exposure decline requiring an updating of preventive measures is necessary.

The purpose of the study was to inform an updated health surveillance scheme for LA workers and adapt preventative measures to the current work conditions. In this respect, major issues were the ascertainment of the current prevalence of LA allergy and the relative role of endotoxin, irritants, and allergens in respiratory and/ or skin symptom generation. To this end, a previously described categorization scheme (Pacheco *et al.*, 2003) for discriminating between endotoxin and allergen exposure was used, care was taken to assess objectively allergens and endotoxin exposure, and a wide range of causes of symptoms was considered. The study was descriptive and the population was working in a university setting.

Materials and Methods

The study was carried out in the frame of an analysis of occupational risks of workers exposed to animals in different units of the faculties of medicine, veterinary medicine, and sciences. It was conducted according to the Declaration of Helsinki and approved by the ethics commission (canton of Zurich; KEK-ZH-Nr. 2012-0142). The study purpose was explained at information meetings, workers received written information, and all subjects gave written informed consent.

The study was made of three consecutive parts (Figure 1).

As the study was planned, no data about work-related animal allergy was available for the studied population. Therefore, a preliminary interview study was

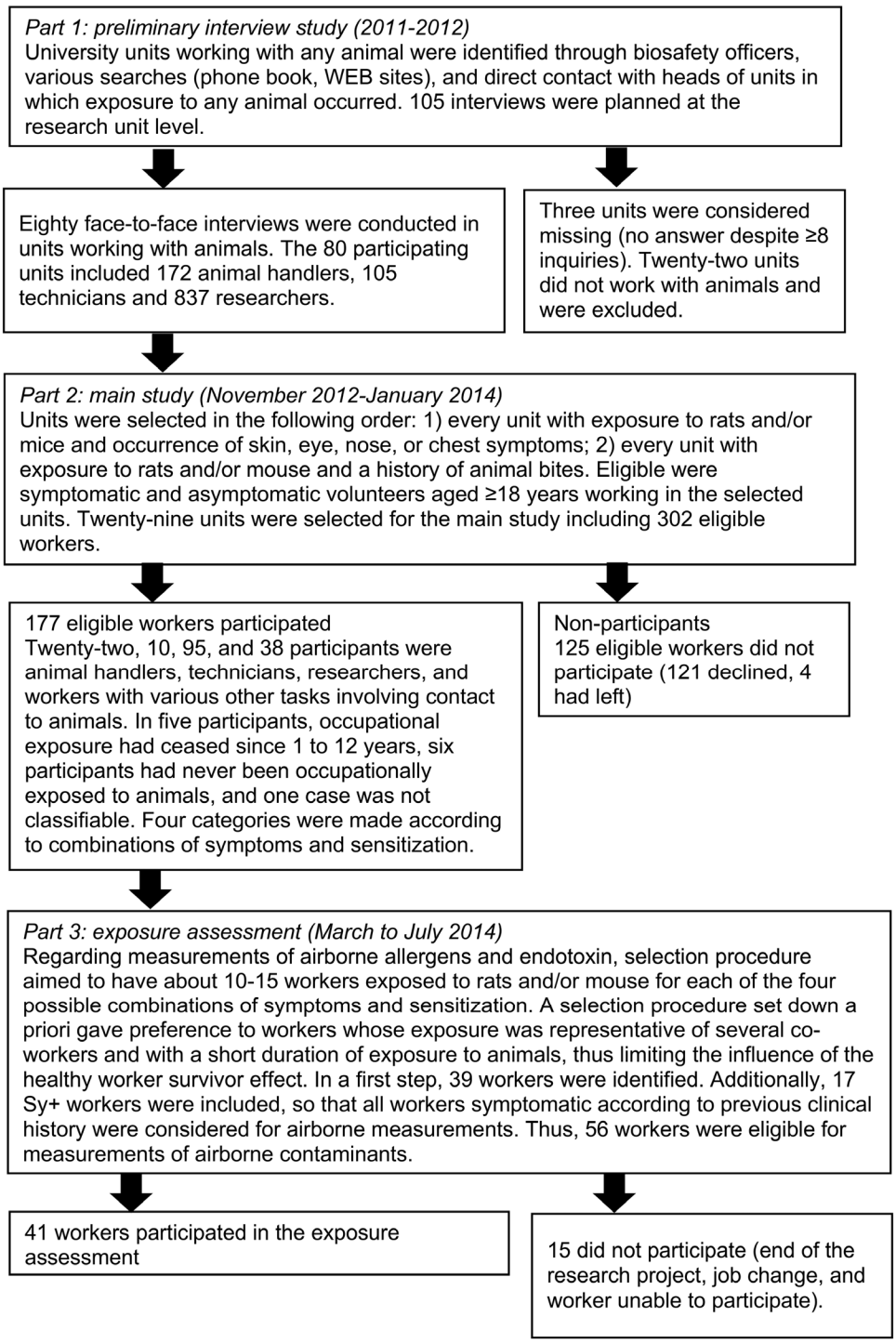

Figure 1.

conducted (2011–2012) at the unit level to gather information on animals, personnel, working conditions, and occurrence of work-related symptoms in anyone working in this unit. This information was reported by the interviewed unit representative.

Regarding the main study (November 2012–Janu- ary 2014), work with exposure to animals other than rodents was assumed to occur in too few subjects for allowing valid conclusions and/or to entail a lower sensitization risk. Therefore, exposure had to include rats and/or mouse for a unit to be selected. However, simultaneous exposure to other animals was not an exclu- sion criterion. (Figure 1). As a lung–skin connection in occupational allergy has been suggested (Redlich and Herrick, 2008; Heederik *et al*., 2012) animal bites were also considered in the selection procedure.

Clinical examination

Clinical examination, spirometry, and IgE (rat- and mouse-specific and total) determinations have been described in details elsewhere (Lemaire et al., submitted). Briefly, clinical and occupational history were recorded through semi-structured interviews by trained physicians using a checklist and written instructions. When symptoms were reported, the worker was asked about their characteristics, concomitant symptoms or signs, and circumstances of occurrence. Cases who were both work-related (WR) and non-WR were considered as WR. Measurements of serum IgE were carried out blinded in batches of coded samples. Total and specific IgE against mouse (e88, Mouse epithelium, serum proteins, and urine proteins) and rat (e87, Rat epithelium, serum proteins, and urine proteins) were measured by ImmunoCAP (Phadia AB, Uppsala, Sweden). Categories of total IgE were used to define atopy with cut-offs of <26 kU l^{-1} (probability of being non-atopic: 84%) and >100 kU l^{-1} (probability of atopy 78 %). Concentrations of total IgE <2 kU l^{-1} were attributed a value of 1 kU l^{-1}. Workers were categorized in four subgroups accord- ing to work-related symptoms and specific sensitization [non-symptomatic and non-sensitized (Sy−/Se−), symptomatic and sensitized (Sy+/Se+), symptomatic and non-sensitized (Sy+/Se^{-}), non-symptomatic and sensitized (Sy−/Se+)] because this classification scheme had been previously considered suitable for discriminating allergen- and endotoxin-induced work-related respiratory and skin symptoms in LA workers (Pacheco *et al*., 2003). According to this classification system, symptomatic (Sy+) individuals were defined as those having ever had work-related symptoms. The pre-defined symptoms systematically assessed in the clinical examination were irritation of conjunctiva, nose or throat, rhinitis symptoms (itchy, runny or stuffy nose, sneezing, and common cold), cough, wheeze; asthma attack, skin rash, itchy skin, and other skin symptoms. Work-relatedness was defined as caused by a specific work task and without non-occupational cause. Regarding irritant effects, the occurrence of similar symptoms in co-workers was also considered. The association between work and symptoms had to be probable or certain as assessed by clinical history. Sensitized (Se+) workers had a specific IgE concentration to rat and/or mouse ≥0.35 kU l^{-1}.

Exposure assessment

Lifelong history of exposure to animals was reconstructed on the basis of a semi-structured interview. First, all successive jobs were recorded in chronological order. Then, the interviewer systematically asked for additional information relating to jobs involving exposure to animals (beginning and end of each employment, job category, cages with high efficiency particulate air filter, and animal bites). If there had been important changes (job, workplace) when working for the same employer, two different forms were filled in to take these changes into account. Source of occupational exposure to animals was classified into five categories: Animal handlers (caring for animals, changing and cleaning cages, changing litter, feeding, and breeding); technicians (collecting blood/urine, surgery, biopsy, administering active ingredients, and euthanasia); researchers (including trainees, students, and any person conducting experimental work); various other tasks with exposure to animals (e.g. veterinarians); no occupational exposure to animals.

Personal sampling (March to July 2014) was used to collect samples in parallel onto two separate filter devices, by means of pocket pumps (SKC pocket pump Air Chek 2000 Model 210–2002, SKC Inc., USA), set at a flow rate of 2.0 l min^{-1} (endotoxin) or 3 l min^{-1} (allergens). Airflow was calibrated before and after field sampling with a piston calibrator (DryCal DC-Lite, Bios International, Pompton Plains, USA). As a rule, sampling was carried out separately in the morning and afternoon. Pumps were not worn during breaks. As duration of exposure varies task-dependently and may be less than a whole 4-h half-day, a minimum duration of 1 h was required. Whether shorter time periods should be sampled had to be decided later according to study results. The mean of the morning and afternoon measurement of each worker and the highest of the individual morning and afternoon measures was defined as the daily and the maximum exposure, respectively. Daily exposure was not computed when only one half-day of sampling had been possible. If only one sampling half-day had been

possible this measurement was considered as maximum exposure.

Total airborne dust for endotoxin assessment was collected onto polycarbonate filters (37 mm diameter, 0.4 micrometer pore size), placed into a ready to use polystyrene closed-faced cassette (endofree-cassette, Aerotech Laboratories, Inc., Phoenix, USA). Total airborne dust for allergen assessment was collected onto gelatin filters (25 mm diameter, 3.0 micrometers pore size, SKC Inc. Eighty Four, PA 15330, USA) placed into a closed-faced polystyrene cassette. After sampling, cassettes were kept in a cold box, transported to the laboratory within the same day and stored at -20°C for 1–3 months to await measurement. Endotoxins were extracted by shaking the filters at room temperature for one hour in 10 ml of pyrogen-free water in a 50-ml conical polypropylene tube. Endotoxins were analyzed using a quantitative kinetic chromogenic Limulus Ameobocyte Lysate assay (Lonza Group, Visp, Switzerland) with an automated microtiter plate reader. *Escherichia coli* O55:B5 endotoxin (Lonza Group) was used as a calibration standard to calculate endotoxin concentration in the experimental samples. Results were expressed in units of endotoxin (EU) m^{-3} air. Rat and mouse allergens were analyzed using ELISA kits (Rat n 1 ELISA kit (EL-RN1); Mus m 1 ELISA kit (EL-MM1), Indoor biotechnologies, Warminster, UK). Allergens were extracted by dissolving the gelatin filter at 37°C during 3 minutes in 1 ml of sterile water in an Eppendorf tube. Then, the protocol proposed by the manufacturer was used. Each sample was measured in duplicate and the mean was used as a result. Results were expressed in ng allergen m^{-3} air. Limits of detection (LOD) were 0.05 EU/filter, 0.097 and 0.024 ng/filter for endotoxin, rat allergen, and mouse allergen, respectively. A concentration equal to half the LOD was attributed to samples below this limit, while two samples with very high concentrations (>25 ng/filter) were attributed a value of 25 ng/filter for statistical analysis. Measurements of airborne contaminants were carried out blinded in batches of coded samples. Results can be compared with those measured by the same laboratory using the same assay in previous studies on respiratory conditions in the same region (Daneshzadeh Tabrizi *et al.*, 2010; Tschopp *et al.*, 2011).

Exposure indicators

As Pacheco *et al.* (2003) critically discussed the limitations of their exposure estimates and suggested that 'verification of these exposure estimates awaits further study', we attempted to reproduce their 'cumulative exposure'. Briefly, Pacheco *et al.* (2003) measured endotoxin concentrations in 11 combinations of specific work tasks and work sites (altogether 43 stationary samples). Then, combination-specific arithmetic means were calculated, extrapolated to all subjects carrying out the same task-site combination, and finally multiplied by the number of hours the subject reported performing the task. All performed tasks were summed and the resulting daily exposure was multiplied by the total number of months in the current job reported by each subject. We used the calculation and extrapolation rules reported by Pacheco *et al.* (2003) but replaced their calculated estimates of daily exposure by the mean daily exposure actually measured in each of the five job categories. The measured mean daily exposure was extrapolated to all workers belonging to the same job category and multiplied by the total number of years in the current job (EU m^{-3} × years).

Statistics and sensitivity analyses

Data on prevalence and incidence of LA allergy show considerable discrepancies (Bakerly *et al.*, 2008; Folletti *et al.*, 2008; Gautrin *et al.*, 2008; Vandenplas *et al.*, 2011) making accurate power calculations impossible. Consequently, we used the most recent study having examined the four possible combinations of symptoms and sensitization simultaneously (Pacheco *et al.*, 2003) to assess crudely how large the population had to be for enabling us to make four subgroups of 10–15 workers according to the four combinations of symptoms and specific sensitization. In the aforementioned study, the percentages were 66, 10, 12–13, and 11–12% in Sy−/Se−, Sy+/Se+, Sy+/Se−, and Sy−/Se+ subjects, respectively. Therefore, if these results were generalizable, even the smallest subgroup was expected to include 10–15 workers if a population of at least 100 subjects was examined.

The study was descriptive. Statistical analysis was performed using SAS 9.4 statistical software (SAS Institute, Cary, North Carolina, USA). Variable distributions were examined for normality, and non-parametric tests or logarithmic transformation used when appropriate (χ^2, Fisher's exact, Wilcoxon two-sample, or Kruskal–Wallis test, analysis of variance). Subjects with a missing value for one variable were excluded from the analyses using this variable. Results <LOD were included in the calculations and were not considered as missing.

The computation of the aforementioned exposure indicators rely on at least two strong assumptions, the use of means and the definition of the time period. Pacheco *et al.* (2003) used the arithmetic mean assuming that 'the arithmetic mean better reflects excursions in exposure that may be important for intermittent symptom generation'. However, owing to the non-normal distribution of exposure measurements medians may be preferred and were used in our sensitivity analyses.

Secondly, with respect to the time period Sy+ individuals were defined as having ever had work-related symptoms, which does not fit in well with a 'cumulative exposure' based on current airborne concentrations and reported number of months in the current job. Therefore, sensitivity analyses were conducted after defining Sy+ as having had symptoms in the last year. For computing the latter exposure indicator the exposure duration was reduced to a maximum of 1 year (less if the worker had worked <1 year). Further sensitivity analyses were conducted using the cut-off of >0.7 kU l^{-1} for defining sensitization, which increases test specificity (Hollander *et al.*, 1996; Heederik *et al.*, 1999).

Results

Part 1: preliminary survey

As expected, the preliminary study showed that mice and rats were by far the most frequent species (58 and 27 units, respectively) but >20 other animal groups occurred as well (mostly sheep in 13 units). Other species occurred less often (for example, cat, horse, rabbit, hamster, dog, or guinea pig in 10, 9, 9, 7, 6, and 5 units, respectively). Thus, exposure was mostly to more than one animal species and a clear-cut separation between mouse and/or rats and other animals was hardly possible. Indeed, a single exposure to mice or rat was found only in 29 and four units, respectively. Eye/nose, skin, and respiratory symptoms in the unit were reported in 17, 7, and 10 interviews, respectively.

Part 2: main survey

Whole population

From 302 eligible workers, 177 participated (58.6%) (Figure 1). One case was partly missing (no attribution to a subgroup was possible). Median age differed between participants and non-participants (31 versus 37 years; $P = 0.006$), whereas gender did not differ statistically significantly ($P = 0.15$). More than 15 different nationalities were represented but only four of them including >10 subjects (Switzerland, Germany, Italy, other) were considered for comparing participants with non-participants ($P = 0.8$). Overall, 41 cases of asthma (occupational and/or non-occupational) were recorded (Table 1). Diagnoses had been confirmed by a doctor in 36/41 (87.8%) workers, while diagnoses were self-reported by five participants. No worker was diagnosed with an organic dust toxic syndrome or hypersensitivity pneumonitis. Fifty-four, 58, 32, 19, 8, 4, 1 workers had worked at 1, 2, 3, 4, 5, 6, and 8 different workplaces. Eight participants had changed workplace for health reasons, four of whom because of allergic disease (1 rhinitis, 3 asthma). Two of them currently had symptoms due to bystander exposure. 52.8% of the 159 workers currently working with animals able to bite reported animal bites. Therefore, bites in workers currently working with animals were much more frequent that sensitization to rat and/or mice, thereby not supporting the hypothesis of a tight association between bite and sensitization to rat and/or mice. At the time of the investigation, only nine of 164 workers with usable answer wore a respirator protecting from organic dust more than 50% of the time. Hence, even in recent years an efficient respiratory protection was seldom worn.

Life-long occupational exposure to animals was associated both with symptoms and sensitization (Table 1) and with job category (median in researchers and technicians of 5 and 17 years, respectively, with values in between in animal handlers and those with various other tasks with exposure to animals; $P < 0.0001$). Atopy (total IgE >100 kU l^{-1}) was associated with the risk of having positive specific IgE against mouse and/or rat (odds ratio (OR) and 95% confidence interval (95% CI): 7.31 (2.05–27.27) ($P = 0.0007$). Having had a cat and/or dog during childhood ($n = 130$) was associated with an OR (95% CI) of 0.44 (0.13–1.53) ($P = 0.2$) of positive specific IgE against rat and/or mouse, while having had a rat and/or mouse ($n = 30$) was associated with an OR of 2.09 (0.51–8.06) ($P = 0.3$) of positive specific IgE against rat and/or mouse. These ORs must be interpreted with great caution as only 14 workers did not report any pet exposure in childhood and 118 subjects reported more than one pet category. The prevalence of Sy−/Se− workers was nearly identical to that expected (68 versus 66%), whereas the two subgroups of Se+ workers included <5% of the population each and were smaller than expected (10–13%). Main population characteristics are presented in Table 1.

Sy/Se subgroups

In the Sy+/Se+ subgroup ($n = 8$), six out of eight workers had a history of rat- and/or mouse-related occupational asthma, required treatment, and had total IgE >100 kU l^{-1} (Table 2). Most of them had other occupational and/or non-occupational allergic manifestations. One case may have been misclassified (irritative dry cough and slightly increased IgE of questionable clinical relevance [e87: 0.35, e88: 2.43 kUA l^{-1})]. Using the cut-off of >0.7 kU l^{-1} for defining sensitization, which increases test specificity (Hollander *et al.*, 1996; Heederik *et al.*, 1999), had no effect on the Sy+/Se+ subgroup. Unexpectedly, life-long occupational exposure to animals was ≤3, 4–5, and >15 years in 2, 1, and 5 workers, respectively, suggesting that recent sensitization was a rare event, which fully

Table 1. Characteristics of the study population

	Group			
	Sy−/Se−	**Sy+/Se+**	**Sy+/Se−**	**Sy−/Se+**
	(*n* = 121)	**(*n* = 8)**	**(*n* = 41)**	**(*n* = 6)**
Age (years)	31.0 (16.0–63.0)	50.0 (27–64)	31.0 (22–58)	39.0 (28–53)
Sex (women)	83 (68.6)	4 (–)	26 (63.4)	3 (–)
Education level				
High	86 (71.1)	7 (–)	26 (63.4)	5 (–)
Middle	34 (28.1)	1 (–)	13 (31.7)	1 (–)
Low	1 (–)	0 (–)	2 (–)	0 (–)
Nationality				
Switzerland	57 (47.1)	4 (–)	20 (48.8)	1 (–)
Germany	24 (19.8)	1 (–)	11 (26.8)	2 (–)
Italy	10 (8.3)	0	1 (–)	0
Other[a]	30 (24.8)	3 (–)	9 (–)	3 (–)
Smoking				
Never	82 (67.8)	5 (–)	31 (75.6)	3 (–)
Former	21 (17.3)	2 (–)	5 (–)	2 (–)
Current	18 (14.9)	1 (–)	5 (12.2)	1 (–)
Pack-years				
(Cigarette smokers only) (*n*)	2.95 (0.05–21) (39)	4.05–35 (3)	3.60 (0.25–22.75) (10)	0.20–5.25 (*n* = 3)
BMI (kg m^{-2})	22.49 (17.31–35.98)	25.2 (19.4–35.3)	23.2 (16.4–35.4)	23.2 (21.5–32.8)
FEV1 (percent predicted)[b]	103.3 (79.2–139.1)	98.5 (84.3–109.1)	102.8 (68.9–136.9)	94.1 (87.4–128.2)
FEV1/FVC (%)[b]	78.4 (53.7–94.9)	70.1 (59.2–80.6)	77.4 (58.2–90.1)	79.3 (71.5–80.5)
Health condition				
Symptomatic[c]				
No	35 (28.9)	0	0	0
Non-WR	86 (71.1)	0	0	6 (–)
WR	0	8 (–)	41 (100)	0
Allergic rhinitis				
No	80 (66.1)	1 (–)	20 (48.8)	5 (–)
NWR	41 (33.9)	0 (–)	10 (24.4)	1 (–)
WR	0 (0)	7 (–)	11 (26.8)	0 (0)
Any allergic disease[d]				
No	58 (47.9)	0 (0)	9 (–)	1 (–)
NWR	63 (52.1)	1 (–)	7 (–)	5 (–)
WR	0 (0)	7 (–)	25 (61.0)	0 (0)
Asthma[e]				
No	99 (81.8)	1 (–)	32 (78.1)	3 (–)
NWR	22 (18.2)	1 (–)	5 (–)	3 (–)
WR	0 (0)	6 (–)	4 (–)	0 (0)
Total IgE (kU l^{-1})	28.1 (1.00–1451)	163.5 (22.5–1209)	30.9 (1.00–1127)	146.5 (18.9–306)
Total IgE				
≤100	100 (82.6)	2 (–)	30 (73.2)	3 (–)
>100	21 (17.4)	6 (–)	11 (26.8)	3 (–)

Table 1. *Continued*

	Group			
	Sy−/Se−	**Sy+/Se+**	**Sy+/Se−**	**Sy−/Se+**
	(*n* = 121)	**(*n* = 8)**	**(*n* = 41)**	**(*n* = 6)**
Specific IgE (≥0.35 kUA l^{-1})				
Rat	0	1	0	0
Mouse	0	1	0	4
Mouse + rat	0	6	0	2
Lifelong occupational exposure to animals (year)	6.0 (0-43.0)	20.5 (3.0-39.0)	7.0 (0-37.0)	10.5 (4.0-32.0)

Values are median and range or number and percent. *n*: sample size. One case is missing (no attribution to a subgroup because of missing values). Percentages not indicated for $n < 10$. BMI, body mass index.

[a]Other nationality' includes >12 different nationalities.

[b]Spirometric results includes only acceptable spirometric curves in Caucasians. Regarding FEV1 [forced expiratory volume in the 1st second (FEV1)/forced vital capacity (FVC)], subgroup size is 98 (95), 7 (6), 37 (36), and 5 (5) in subgroups Sy−/Se−, Sy+/Se+, Sy+/Se−, and Sy−/Se+, respectively.

[c]Symptomatic', 'non-work-related (NWR)' and 'work-related' (WR): see Methods. Note that according to the classification scheme subjects having any pre-defined symptom are considered asymptomatic when the symptom is not work-related. Cases who were both WR (work-related) and non-WR were considered as WR.

[d]Any allergic disease comprises allergic rhinitis, asthma, eczema, or any other skin allergy, and allergy to animals.

[e]Asthma (occupational and/or non-occupational) was defined by a positive response to the question 'Have you ever had asthma'? Asthma had been confirmed by a doctor in 36/41 (87.8%) workers.

agrees with information from clinical history (Table 2). No comparison between those having been exclusively exposed to mice and/or rats and those exposed to mice and/or rats and other animals could be done (limited sample size, few workers with a lifelong exposure to rat and/or mice only, confounding by non-occupational exposure to pets).

In the Sy−/Se+ subgroup ($n = 6$), no common characteristic of the six workers could be identified. Using the stricter cut-off of >0.7 kU l^{-1} would have moved two of the six workers to the Sy−/Se− subgroup.

The Sy+/Se− subgroup ($n = 41$) was largely similar to the Sy−/Se− group. Indeed, apart from the number of symptomatic workers and subjects with allergic rhinitis, asthma, and any allergic disease, the variables listed in Table 1 did not differ statistically significantly between subgroup Sy−/Se− and Sy+/Se− ($0.15 < P < 0.9$). Moreover, a comparison of symptoms unlikely to be associated with occupation (depressed without any reason, became irritated without any reason, appetite problems apart from loss of appetite, or irregular bowel movements) between the Sy−/Se− and the Sy+/Se− groups did not suggest systematic symptom over-reporting in the subgroup Sy+/Se− ($0.2 < P < 1.0$). Total duration of exposure to animals was always >3 years.

Contrary to expectations, endotoxin did not prove to be a major cause of symptoms in this Sy+/Se− subgroup, and on the basis of occupational and clinical history various other reasons for being 'symptomatic' were identified. Indeed, in only three workers work conditions and clinical history were clearly compatible with an endotoxin-induced irritant effect resulting from a task with exposure to organic dust, whereas nine workers had an obvious irritant reaction due to cleaning agents/disinfectants (e.g. hydrogen peroxide vapor), formaldehyde (e.g. pathology, disinfection), or glove-induced irritation. Nineteen workers had a clinical history of allergic diseases often suggesting other allergens than those from rat and/or mouse (e.g. cow, horse, cat, dog, rabbit, latex, plant allergens) or had taken measures to reduce or prevent exposure to rat and/or mouse allergen. Indeed, 12 workers had been occupationally asymptomatic in the last year. A few cases were likely to be symptomatic because of a type IV allergy (gloves). In six cases clinical history was compatible with both an allergic and an irritant reaction and four cases clearly reported work-related complaints related neither to irritant nor to allergen. Overall, in this Sy+/Se− group occupational and clinical history did not point to endotoxin as a major factor for symptoms without sensitization but disclosed numerous different causes of symptoms.

Part 3: exposure assessment

Airborne allergens and endotoxin

Regarding measurements of airborne allergens and endotoxin, Sy+/Se− workers were overrepresented ($n = 20$; 48.8%) as aimed at by the selection proce- dure. Measurements were performed on 31 different days over five months (March–July 2014). The two half-day sampling periods were from the same day

Table 2. Main characteristics of the Sy+/Se+ subgroup

Current job category/ duration (year)	Previous job category with animal exposure /duration (year)	Lifelong exposure to animals (years)	Symptoms treatment	Clinically rat- or mouse-related asthma	FEV1 (percent predicted) FEV1/FVC (%)	Total IgE (kU l^{-1}) e87 (kUA l^{-1}) e88 (kUA l^{-1})	Endotoxin concentrations (morning/afternoon) (EU m^{-3}) Others
Researcher/4	None	4	N, R AH, BA, S B. 1990	Yes	95.06 NA	Total: 162.0 e87: 5.18 e88: 28.80	NA/NA Direct and bystander exposure causes symptoms
Researcher/4	Researcher/8 Researcher/6 Researcher/2 Researcher/1 Researcher/4	25	E, N, R AH B: 1991	Yes	98.46 71.93	Total: 165.0 e87: 1.00 e88: 11.50	8.3/10.3 RPE use Asthma was exclusively occupational
Research manager (exposure stop in 2005)	Researcher/16	16	E, N, R AH, BA, S B: 1988	Yes	84.26 68.16	Total: 79.60 e87: 0.05 e88: 2.34	1.1/2.7 Had to stop exposure because of allergy but still symptomatic in lift when animals are transported
Researcher/4	Researcher/3 Researcher/8 Researcher/16	31	E, N, R, U AH, BA, S B: 1998	Yes	89.08 59.22	Total: 1209.0 e87: 6.91 e88: 12.0	NA/NA
Researcher/3	None	3	E, N, R AH, BA B: 2010	Yes	107.18 75.31	Total: 426.0 e87: 14.70 e88: 2.34	3.6/15.3 RPE use
Animal handler/33	Miscellaneous exposure/1 Researcher/5	39	R (infrequently) No antiallergic treatment B: unknown	Very unlikely given the clinical picture	NA	Total: 419.0 e87: 0.35 e88: 2.43	11.0/9.9 Non-acceptable spirometry curves
Technician/34	None	34	No symptom in the last year T, BA, S B: 1990	Yes	107.79 59.62	Total: 22.50 e87: 2.41 e88: 0.08	NA/NA Asthma was exclusively occupational
Researcher/3	None	3	No symptom in the last year No antiallergic treatment B: 2013	No asthma but U and N	109.07 80.63	Total: 132.0 e87: 2.39 e88: 0.54	0.3/0.2 RPE use. Has to limit long or intense exposure

Job category: see 'methods' Job categories listed from the most recent to the oldest one. AH, antihistamine; BA, beta agonist; B, year of beginning of symptoms of occupational allergy recorded in 2013; E, eye; NA, not available; N, nose; S, steroid; T, theophylline; RPE, respiratory protection equipment; R, respiratory; U, urticaria; e87 and e88, specific IgE against rat and mouse, respectively.

(n = 30) or from two different days (n = 8; 1–7 days between the 2 sampling periods). Three workers had measurements during half a day only. Median (range) morning sampling time was 200 min (106–305) and 200 min (108–292) for allergen and endotoxin, respectively. Median (range) afternoon sampling time was 176 (84–253) and 173 (62–254) min for aller- gen and endotoxin, respectively. Sampling times were quite comparable in the four subgroups (0.5< P <1.0). Median temperature (°C, range) was 23.3 (19.3–26.5) and 23.6 (21.1–27.1) in the morning and afternoon, respectively. Median humidity (%, range) was 37.9 (27–59.4) and 36.7 (23.8–64.7) in the morning and afternoon, respectively.

Rat allergen was ≥LOD in four and six morning and afternoon samples, respectively (Table 3), but six samples were exactly equal to the LOD making statistical calculations impossible. Mouse allergen was ≥LOD in 51.9% of the samples. Regarding endotoxin, 41 and 38 morning and afternoon samples were collected with only one sample <LOD. Mouse allergen and endotoxin concentrations varied widely (Table 3).

No statistically significant difference in mouse allergen and endotoxin concentrations was found between morning and afternoon samples (signed rank test; P > 0.60 for mouse allergen; P > 0.10 for endotoxin) allowing for computation of daily concentrations. Daily and 'maximum' concentrations are indicated in Table 3. With respect to endotoxin, all morning concentrations (n = 41), apart from four, were <20 EU m^{-3} (77.3, 110, 390 and 1659 EU m^{-3}), while three of 38 afternoon concentrations exceeded this value (20.2, 62.9, and 251.3). Thus, 72/79 half-day concentrations were <20 EU m^{-3}). Use of straw, cleaning and feeding tasks, and contact with large animals (pig, sheep) were associated with the two highest endotoxin concentrations (390 and 1659 EU m^{-3}) (see Online Supplementary Material). Differ- ences between subgroup endotoxin concentrations were of borderline significance (Table 3).

Cumulated exposure

The calculated cumulative endotoxin 'exposure' did not differ between the Sy−/Se− and Sy+/Se− subgroups [median (range): 59.48 (0-3621) versus 29.74 (0-2769) EU m^{-3} × years, respectively; P = 0.99]. Owing to the prevalence of results <LOD, cumulative exposures were not computed for rat and mouse allergen.

Sensitivity analyses

With respect to the time period of symptom occurrence, substituting 'past year' for 'ever' moved 14 (7.9%) workers into another category of symptoms and sensitization (133, 6, 29, and 8 Sy−/Se−, Sy+/Se+, Sy+/Se−, Sy−/Se+ workers). As a consequence, the significance level of the difference in endotoxin daily and maximum concentrations between subgroups Sy−/Se− and Sy+/Se− lessened considerably (Table 4).

Sensitivity analyses pertaining to the selection of the median instead of the mean showed that the cumulative exposure computed for a worker heavily depended on the choice of the parameter estimate (median or mean). Indeed, the median daily endotoxin concentration was 0.83 and 2.97 EU/m^3 in the group 'researchers' (n = 16) and 'various other tasks with exposure to animals' (n = 13), respectively. Thus, the cumulative exposure for a hypothetical worker with a 10-year duration of exposure was 8.3 and 29.7 EU m^{-3} × year, respectively. Arithmetic means were 29.74 and 4.30 EU m^{-3} in the group 'researchers' and 'various other tasks with exposure to animals', respectively, resulting in cumulative exposures of 297.4 and 43.0 EU m^{-3} × year for the two same groups of workers. Thus, a difference of one order of magnitude arose and the ranks of the two workers were inverted. Similar results were found when calculating cumulative exposures based on maximum exposure measurements (details notshown).

Discussion

The study assessed the current exposure to airborne allergens and endotoxin in a population working in a university setting and exposed to a range of animals besides laboratory mice to serve as a basis for updated surveillance and prevention measures. Results showed low airborne concentrations of allergens and endotoxin. Moreover, while 49 subjects were symptomatic (Sy+), only eight of them were both symptomatic and sensitized.

The low allergen and endotoxin levels and the low prevalence of sensitization, especially in workers with <4 years of lifelong occupational exposure to animals (Table 2), must be interpreted with caution owing to the lack of longitudinal data. However, the two findings may result from the increased use of genetically modified mice and the introduction of IVCs (Jones, 2015; Feary and Cullinan, 2016). This issue is of importance for prevention. Indeed, as exposure to rat and/or mouse allergen declines, the relative importance of other occupational allergens, irritants, PAMPs, and/or individual susceptibility could increase. Findings from the occupational history support this interpretation. Indeed, other allergens than those from rat and/or mouse (e.g. other animal's allergens, latex or plant allergens), cleaning agents/disinfectants, and type IV reaction caused symptoms in much

Table 3. Exposure measurements

	Subgroup				
	Sy−/Se−	**Sy+/Se+**	**Sy+/Se−**	**Sy−/Se+**	***P* value (Sy−/Se− versus Sy+/Se−)**
Rat allergen <LOD (ng/filter) (*n*)					
Morning (*n* = 41)	10/11	4/5	18/20	5/5	1.0
Afternoon (*n* = 38)	8/9	5/5	15/19	4/5	1.0
Mouse allergen <LOD (ng/filter) (*n*)					
Morning (*n* = 41)	6/11	0/5	13/20	1/5	0.7
Afternoon (*n* = 38)	5/9	1/5	11/19	1/5	1.0
Daily exposure (ng/m^3) (*n* = 35)	0.02–12.69 (7)	0.03–50.94 (5)	0.03 (0.02–9.97) (18)	0.03–7.52 (5)	0.4
Maximum exposure (ng/m^3) (*n* = 40)	0.64 (0.02–17.03) (10)	0.04–50.95 (5)	0.03 (0.02–18.10) (20)	0.03–14.75 (5)	0.4
Endotoxin (EU/m^3)					
Morning (*n* = 41)	0.54 (0.24–4.58) (11)	0.25–11.02 (5)	1.29 (0.17–390) (20)	0.32–1659 (5)	0.2
Afternoon (*n* = 38)	0.15–4.31 (9)	0.21–15.34 (5)	1.83 (0.29–251) (19)	0.09–20.2 (5)	0.09
Daily exposure (*n* = 38)	0.23–2.84 (9)	0.23–10.46 (5)	1.99 (0.30–320) (19)	0.20–839 (5)	0.06
Max. exposure (*n* = 41)	1.44 (0.32–4.58) (11)	0.25–15.34 (5)	2.56 (0.17–390) (20)	0.32–1659 (5)	0.09

Results are number or median, range, and number (median not indicated when size <10). Subgroup size may slightly vary because of participant non-available for one half-day (*n* = 3) or pump malfunction. LOD, limit of detection (expressed in ng/filter; see 'Methods'). Regarding mouse allergen, a concentration equal to half the LOD was attributed to samples <LOD, while two samples with very high concentrations (>25 ng/filter) were attributed a value of 25 ng/filter for statistical analyses. Only one endotoxin sample was <LOD.

P value (Kruskal–Wallis test) for comparison between subgroups Sy−/Se− and Sy+/Se− only (owing to the small subgroup size results from subgroups Sy+/Se+ and Sy−/Se+ are given only for the sake of completeness).

Table 4. Effect of the time period selected for outcome assessment on the endotoxin concentration (EU m^{-3})

	Subgroup		*P* value
	Sy−/Se−	Sy+/Se−	
Time period of symptom occurrence 'ever'			
Daily exposure (*n*)	0.23–2.84 (9)	1.99 (0.30–320) (19)	0.06
Maximum exposure (*n*)	1.44 (0.32–4.58) (11)	2.56 (0.17–390) (20)	0.09
Time period of symptom occurrence 'past year'			
Daily exposure (*n*)	1.25 (0.23–320) (17)	1.58 (0.30–86.4) (11)	0.6
Maximum exposure (*n*)	1.72 (0.32–390) (19)	1.89 (0.17–110) (12)	0.8

Time period for symptom occurrence is 'ever' (top) and 'past year' (bottom). Subgroup size may slightly vary because of participant non-available for one half-day ($n = 3$) or pump malfunction.
P value according to Kruskal–Wallis test.

more workers than specific sensitization to rat and/or mice (41 versus 8 workers).

A simple classification scheme would be extremely useful for efficiently identifying workers with endotoxin-induced symptoms. However, although every effort was made to reproduce the method used by Pacheco *et al.* (2003), we were unable to reproduce their finding of higher endotoxin exposure in the Sy+/Se− subgroup, although measured endotoxin concentrations were similar in both studies. Indeed, the highest concentration these authors measured amounted to 1 463 pg m^{-3} (≈14.63 EU m^{-3}), which compares well with those of the present study (72/79 half-day concentrations <20 EU m^{-3}) and those reported by Lieutier-Colas *et al.* (2001) [all but two concentrations below 10 ng m^{-3} (≈100 EU m^{-3}), $n = 242$]. The discrepancy between the results of these studies could result from the use of different methods for estimating the 'daily' exposure. Indeed, in the study by Pacheco *et al.* (2003) arithmetic means representing 11 combinations of major task and work site (overall, 43 stationary measurements with 2–7 measurement for each combination) were used to compute the 'daily' exposure of 269 workers, while we objectively assessed it with personal samples. Moreover, the 'cumulative exposure' resulted from multiplying the computed 'daily exposure' by the number of reported months in the current job and could not be representative of individual and variable long-term exposures. In contrast, in rat-exposed workers with an extensive exposure assessment [128 stationary samples for estimating the daily exposure in 113 workers; endotoxin concentrations of personal and stationary endotoxin samples well correlated ($r = 0.88$; $n = 38$)] endotoxin did not seem to be important in the generation of rat-related symptoms (Lieutier-Colas *et al.*, 2001; Lieutier-Colas *et al.*, 2002). Of note, an association between intensity of endotoxin exposure and symptoms is not quite consistent with the dose–response relationship. Indeed, all concentrations were well below the currently suggested occupational guidance values of 90–1000 EU m^{-3} (Nordic Expert Group and Dutch Expert Committee, 2011; Duquenne *et al.*, 2013; Samadi *et al.*, 2013; Feary and Cullinan, 2016) (Further details in Online Supplementary Material). As the computation of the exposure surrogates rely on at least two strong assumptions (means and time period) great attention should be given to control biases and confounders capable of affecting them.

Regarding the role of allergens, it should be stressed that the LOD of the assays we used were not higher than those of recent publications (Pacheco *et al.*, 2006; Curtin-Brosnan *et al.*, 2010; Glueck *et al.*, 2012; Raulf *et al.*, 2014).

Regarding association between exposure and outcome, time period of outcome occurrence ('ever'), and exposure measurements (one time point) are incongruent. Work conditions have improved over time and research may involve intense exposure for some months, followed by little to no exposure. Consequently, our sensitivity analyses using more congruent time periods should have improved specificity thereby strengthening the association between endotoxin and symptoms. However, the opposite was found suggesting a bias rather than a true association.

Last but not least, a major reason possibly explaining some discrepancies is the selection procedure. Pacheco *et al.* (2003) examined only symptoms and sensitization to mice, while Lieutier-Colas *et al.* (2002) exclusively included rat-exposed workers. We broadened eligibility criteria, which is more representative of exposure settings where many workers are exposed to several animal species.

This study has limitations. As no data was available, it was designed to give baseline information on the current situation. Therefore, the design was descriptive, airborne peptidoglycan and fungi were not considered, and immunological investigations were limited to two specific IgE determinations. Secondly, the participation was 58.6% so that a selection bias may have arisen. Moreover, workers with symptoms and/or risk factors were overrepresented (Figure 1). This was absolutely justifiable to set preventive priorities but limits generalization. Nevertheless, despite these limitations the high proportion of Sy+ workers with a clinical history of condition not due to rat or mouse allergen and the low proportion of sensitizations in those with short exposure duration bears on risk assessment and should be considered when updating surveillance and prevention programs.

Conclusion

The study suggests that prevention programs should not be restricted to one single specific allergen but consider both irritants and a broad range of sensitizers. Including in the surveillance program a classification scheme based on the combination of one single IgE determination and having ever had predefined work-related symptoms represents an attractive approach but may be an oversimplification and underestimate the prevalence of occupational allergy. As suggested by Feary and Cullinan (2016) and Jones (2015), the low levels of exposure to allergens and endotoxin may result from recent changes in the work environment of LA workers. However, this finding has to be confirmed in further studies.

Supplementary Data

Supplementary data are available at *Annals of Work Exposures and Health* online.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Research involving human participants: The study was conducted in the framework of an analysis of occupational risks of workers exposed to animals. It was conducted according to the Declaration of Helsinki and approved by the ethics commission (canton of Zurich; KEK-ZH-Nr. 2012-0142).

Informed consent: The study purpose was explained at information meetings, workers received written information, and all subjects gave written informed consent.

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